

CYPRIPEDIUM FASCICULATUM (ORCHIDACEAE) ANTHESIS AND FRUIT SET IN RELATIONSHIP TO DIAPRIID ACTIVITY

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ABSTRACT. We investigated the pollination biology of a rare endemic lady's slipper orchid, *Cypripedium fasciculatum*, in southwestern Oregon from 1998 to 2001. In 1999, a parasitic wasp (Order Hymenoptera, Family Diapriidae, *Cinetus* sp.) was identified as the pollinator. Understanding the relationship between pollinator activity and floral development is imperative to successfully protect rare plants. We report here on our efforts to characterize the floral phenology of *C. fasciculatum* and to determine the relationship between pollinator activity and *C. fasciculatum* anthesis and fruit set. During the 1998–2001 period, we monitored 916 *C. fasciculatum* flowers in three sites for selected floral events from emergence to fruit set and monitored pollinator activity in the vicinity of the orchid sites using sticky traps. Pollinator activity and fungus gnat (sciarids and mycetophilids) activity, the host insect of the pollinator, was calculated by determining the number caught per trap per day across field sites and years. Our research characterized the floral phenology of *C. fasciculatum* to six distinct stages, with anthesis occurring in stage 2 and 3. Anthesis in *C. fasciculatum* was synchronized with peak diapriid activity as well as peak fungus gnat activity. When diapriid activity was low, then fruit set was low, although the correlation was not significant. A marginal negative correlation was found between diapriid activity and the duration of anthesis. Fungus gnats, particularly sciarids, were the major component of the insect fauna in the sites. Female diapriids were the only insects found vectoring the pollinia of *C. fasciculatum*. We believe the non-rewarding *C. fasciculatum* flowers emit odors that mimic the odor of fungus gnats luring female diapriids to enter a flower in search of a host to parasitize. Trapped diapriids encounter the stigma of *C. fasciculatum* en route to the small posterior opening in the labellum where the pollinia is deposited as the diapriid exits.

Key words: *Cypripedium fasciculatum*, pollination, diapriid wasps, anthesis, fruit set

INTRODUCTION

An estimated 25,000 species of orchids (Orchidaceae) occur worldwide (Dressler 1990). Orchids exhibit diverse pollination mechanisms (Proctor et al. 1996), and many orchids that require or benefit from pollinators do not reward floral visitors (Van der Pijl & Dodson 1966). The reproductive success of non-rewarding orchids, therefore, depends on the orchid's ability to deceive potential pollinators into visiting the orchid's flowers (Neiland & Wilcock 1998). Most non-rewarding orchids exploit a pollinator's general foraging behavior for nectar by displaying vivid colors and/or odors (Ackermann 1981, Nilsson 1992, Lipow et al. 2002). Some orchids ensure pollination by temporarily trapping and attaching pollen to insects that incidentally

visit their flowers (Dressler 1990). Although most North American orchids rely on insects for pollination, pollination mechanisms and breeding systems are described for only about half of the known members of the subfamily Cypripedioideae (Catling & Catling 1991, Doherty 1998).

The clustered lady's slipper orchid, *Cypripedium fasciculatum* Kellogg ex S. Watson is endemic to the western United States (Coleman 1995), where it occurs from northern California north to Washington and east to Idaho, Montana, Wyoming, Utah, and Colorado (Brownell & Catling 1987, Coleman 1995). Populations of *C. fasciculatum* are usually small (each containing less than 20 aerial stems) and scattered across its range (P. Latham & J. Tappeiner unpubl. data). Partly because of the disjunct distribution of *C. fasciculatum*, the USDA Forest Service and the U.S. Department of Interior, Bureau of Land Management, lists *C. fasciculatum* as a

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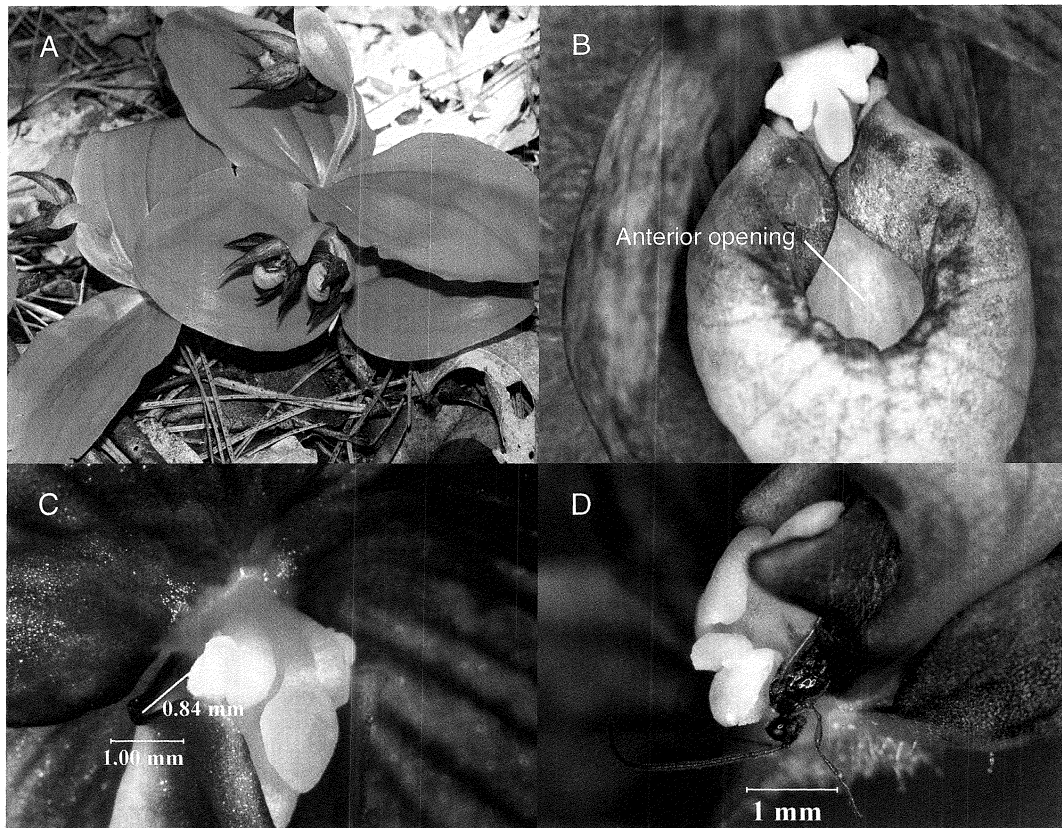


FIGURE 1. Clustered lady's slipper orchid, *Cyripedium fasciculatum* (Orchidaceae), **A.** Drooping flowers clustered at the tip of the peduncle. **B.** Anterior opening of a *C. fasciculatum* flower. **C.** Posterior opening of the lip of a *C. fasciculatum* flower. **D.** Diapiid wasp exiting through the posterior opening of a *C. fasciculatum* flower with pollenia attached. Photos by C. Ferguson.

species of concern (M. Winter pers. comm). In Oregon, *C. fasciculatum* is listed as critically imperiled by the Oregon Natural Heritage Program (2001).

Cyripedium fasciculatum plants, which measure less than 18 cm from the base of the plant to the top of the flowers, are the smallest of the three western *Cyripedium* species that also include *C. montanum* Douglas ex Lindley and *C. californicum* A. Gray. Flowers of *C. fasciculatum* droop at the tip of a peduncle, and each plant may possess a single flower or, more often, a cluster of up to eight flowers (Harrod 1994, Coleman 1995) (FIGURE 1A). The flowers of *C. fasciculatum* measure less than 4.5 cm from tip to tip, and the anterior opening to the lip measures only ca. 7 mm across its widest axis and 4 mm across its narrowest axis (Coleman 1995, C. Ferguson pers. obs.) (FIGURE 1B). Cribb (1997) thoroughly describes the morphology of *C. fasciculatum*.

Since 1998, we have investigated the polli-

nation biology of *Cyripedium fasciculatum* in southern Oregon. Bees, currently recognized as pollinators of *Cyripedium* species other than *C. fasciculatum*, include the families Andrenidae, Apidae, Anthophoridae, Halictidae, and Megachilidae (Stoutamire 1967, Kipping 1971, Nilsson 1979, Catling & Knerer 1980, Plowright et al. 1980, Davis, 1986). In addition, one fly species (Syrphidae) pollinates *C. reginae* (Vogt 1990). The pollinator of *C. fasciculatum*, however, recently was shown to be an undescribed diapiid wasp species (*Cinetus* sp., Diapriidae, subfamily Belytinae) (Ferguson & Donham 1999). Diapriidae is a cosmopolitan family with more than 300 described species of small (3–4 mm long) sting-less parasitic wasps, whose hosts are likely the larvae or pupae of fungus gnats (Mycetophilidae and Sciaridae) (Gauld & Bolton 1988, Masner 1995). Diapriids have been observed on a number of occasions inside the lip of *C. fasciculatum* flowers (K. Donham, P. La-

tham, M. Lee, S. Nyoka, B. Tong pers. comm., Lipow et al. 2002).

Cypripedium fasciculatum offers no nectar reward to insect visitors, suggesting pollinators are attracted by deception (Vogt 1990, Cribb 1997). Insects enter the flower through the anterior opening in the lip, whose recurved margin prevents them from escaping via the same route. Only insects that can squeeze through a small posterior opening or sinus (less than 2 mm in diameter) at the base of the column can escape the flower (FIGURE 1C). Insects first crawl under the stigmatic surface, where pollen (if present) is removed from their body by fine papillae. The insect then squeezes out the posterior opening as it passes underneath one of two fertile anthers that deposit pollen on the dorsum of the insect's thorax. Although we have never observed a diapiiid entering a *C. fasciculatum* flower (even after hundreds of hours of field observations), we have observed on five occasions a diapiiid exiting the posterior opening of *C. fasciculatum* with *C. fasciculatum* pollinia attached to its mesothoracic notum (FIGURE 1D).

Although the arrangement of the flower's reproductive structures increases the likelihood of cross-pollination in *C. fasciculatum*, the small size of the posterior openings restricts which insects can vector its pollen (Proctor et al. 1996). *Cypripedium fasciculatum* is self-compatible but not autogamous or apomitic (Knecht 1996, Lipow et al. 2002), suggesting pollen vectors are needed for successful cross-pollination and good fruit set (Stoutamire 1967, Barth 1991, Knecht 1996). Fruit set in non-rewarding North American orchid species is typically low and averages only 20% (Neiland & Wilcock 1998). Barker (1983) indicated that *C. fasciculatum* also has low fruit set and believed pollination was an infrequent event. More recent studies, however, report a range of fruit set in *C. fasciculatum* populations, such as 18% in Colorado and 29% in Idaho (Lipow et al. 2002), 31% in Oregon (Harrod 1994), 32% in Washington (Knecht 1996), and 69% in Oregon (Lipow et al. 2002).

Rare plants that depend on insect pollination require adequate populations of pollinators (Boyd 1994). Low fruit set in non-rewarding orchids may result from insufficient pollen load or pollinator limitation. An understanding of the pollination systems of rare plants and, more specifically, the relationship between pollinator populations and pollination is limited and deserves more study (Catling & Catling 1991, Buchmann & Nabhan 1996, Kearns & Inouye 1997). No studies to date have investigated the relationships between pollinator populations and percentage of fruit set in *Cypripedium fasciculatum*. Successful efforts to protect rare plants,

such as *C. fasciculatum*, from extinction may rely on an understanding of the relationship of pollinator activity to the plant's floral phenology (the timing of developmental events). Therefore, this study sought to characterize the floral phenology of *C. fasciculatum* and to determine the relationship between diapiiid activity and *C. fasciculatum* anthesis and fruit set. In addition, the activity of diapiiids, as well as the insect's hosts (mycetophilids and sciarids), was monitored before, during, and after orchid bloom in the vicinity of *C. fasciculatum* populations. The goal was to better understand how life history patterns (emergence periods, peak activity periods) of the diapiiids relate to its hosts.

MATERIALS AND METHODS

During each year from 1998 to 2001, research was conducted at two sites in southwest Oregon in forests managed by the United States Department of Interior, Bureau of Land Management. In 1998, 1999, and 2000, research was conducted at the following sites: (1) Murphy Gulch (42°32'09"N, 123°04'03"W), a mixed forest of predominately *Pseudotsuga menziesii* var. *menziesii*, *Arbutus menziesii*, and *Quercus garryana* at 792 m elevation with a southwest aspect containing ca. 200 *Cypripedium fasciculatum* plants. (2) Alexander Gulch (42°10'06"N, 123°09'03"W), an open conifer forest of predominately *Pseudotsuga menziesii* at 1158 m elevation with a steeply sloped (50%) western aspect containing 125 *C. fasciculatum* plants. In 2001, research also was conducted at (1) Murphy Gulch (described above) and (2) Butte Falls (42°34'52.6"N, 122°36'30"W), an open conifer forest of predominately *Pseudotsuga menziesii* and some *Pinus lambertianna* at 626 m elevation with a minimally-sloped north-south aspect containing a crowded population of ca. 100 *C. fasciculatum* plants (most plants occurring in a 3 m by 2 m area).

Floral Phenology

In 1998, a total of 53 flowers on randomly selected *Cypripedium fasciculatum* plants at southern Oregon orchid sites were monitored for selected floral events (number of flowers, appearance of floral structures, appearance and presence of pollen) from when flower buds appeared to when fruit set. To allow for repeated observations of the flowers, an aluminum tag was secured in the ground next to each monitored plant. Each plant was examined weekly using a 10× hand lens, and the phenological stage of the flowers was assessed (described above) along with the presence of any floral

TABLE 1. Phenological stages of *Cypripedium fasciculatum* flowers.

Stage	Floral description
One	Perianth closed, reproductive structures not visible
Two	Sepals reflexed, labellum visible, staminode below base of collar, staminode green, flower in anthesis, pollen yellow and visible
Three	Labellum lower and staminode above collar base, staminode faded to light green, flower in anthesis, pollen yellow (if present)
Four	Sepals and labellum wilted, staminode well above collar base, staminode yellow to white, pollen darkened (if present)
Five	Perianth dry, labellum deteriorated
Six	Perianth abscission, ovary fertile (enlarged and green) or ovary infertile (small and brown)

damage. Floral events then were used to differentiate the bloom period into six phenological stages (TABLE 1). A total of 244, 208, and 411 flowers were examined, in 1999, 2000, and 2001 respectively, and the proportion of flowers in anthesis calculated. Flowers that were still enclosed in the perianth (no exposed reproductive structures) were characterized as stage 1. Stage 2 flowers contained reflexed sepals, a visible lip, yellow pollen visible on the two functional anthers, and a green staminode, whose tip was below the base or collar of the lip. The lip of stage 3 flowers was slightly lower than the lip of stage 2 flowers (causing the tip of the staminode to be positioned above the base of the lip), pollen was yellow, and the staminode appeared light green to yellow. Both stage 2 and stage 3 flowers were in anthesis and receptive to pollinators, but stage 3 flowers may be more accessible to visitors than stage 2 flowers because of the slightly lowered lip. In stage 4 flowers, both the sepals and lip were wilted, pollen (if present) was brown, and the entire staminode, which appeared yellow or white, was positioned well above the base of the lip. Stage 5 flowers possessed a dry perianth and a deteriorated lip. The calyx was sometimes observed closed over the lip in stage 4 and stage 5 flowers. In stage 6, the entire perianth was abscised and the condition of the ovary was assessed.

Flowers in stage 6 were examined for swollen ovaries. Plants were again examined in August for seed capsules. Because only flowers that possessed green swollen ovaries later produced seed capsules (flowers with small brown ovaries did not produce seed capsules), flowers with noticeably swollen green ovaries at the end of the bloom period were considered fertilized (fruit set). Overall rate of fruit set was calculated by dividing the number of fertile ovaries by the total number of flowers monitored in each site. Because overall fruit set does not account for flowers lost to insect or animal browsing or mechanical damage from handling, pollination rates

were underestimated. Thus an adjusted overall fruit set was calculated by dividing the number of fertile ovaries by the total number of mature, viable flowers present at the end of bloom.

Pollinator Activity and Floral Phenology

To assess general insect activity within the sites, insects that were observed visiting or in the proximity of *Cypripedium fasciculatum* plants and flowers were collected using a standard insect aspirator or aerial net. Small flying insects were monitored continuously at each site using sticky traps—7.6 cm by 12.7 cm yellow plastic cards coated on both sides with an odorless sticky substance (Tanglefoot®). Traps were positioned vertically in a metal holder ca. 15 cm off the ground adjacent to, but not in direct contact with, marked *C. fasciculatum* plants. A stereo dissecting scope (Zeiss® Stemi 2000) was used to examine the traps, which were collected weekly, for insects. Trapped insects were identified to family and those carrying *C. fasciculatum* pollen were stored in 90% ethanol or left on the sticky trap and stored in a permanent reference collection at the Southern Oregon University (SOU) Insect Museum.

In 1999, 2000, and 2001, the activity of diapiids and their insect hosts (sciarids and mycetophilids), calculated by determining the number caught per trap per day, was compared across field sites and years. To determine whether a relationship exists between diapiid activity and *Cypripedium fasciculatum* anthesis and fruit set, wasp activity was compared, using linear regression, with duration of anthesis (calculated as the number of days during the orchid's bloom that at least 50% of the flowers were in anthesis) and percent fruit set. Fruit set was compared across years and sites in 1999 and 2000.

In 1999 and 2000, insect traps were employed (as described above) at Murphy Gulch and Alexander Gulch during orchid bloom. All traps were collected and replaced weekly with fresh

traps. In 1999, eight traps were used per sample period in each site, and four traps were used per sample period in 2000. In 2001, to better understand emergence periods and peak activity periods of diapiiids and their fungus gnat hosts, we monitored the activity of diapiiids and fungus gnats during a 12-month period in Murphy Gulch and Butte Falls. Twelve traps were placed in each of two locations relative to each orchid site. One set of traps was placed inside each orchid site while the other set was placed outside each orchid site at a minimum distance of 2 m from the nearest *Cypripedium fasciculatum* plant. The mean number of diapiiids and fungus gnats captured per day was calculated during the 12-month time period.

To understand how effective diapiiids were in pollinating *Cypripedium fasciculatum*, diapiiids were excluded from ten plants at each site (31 flowers in Murphy Gulch and 22 flowers in Alexander Gulch), by placing a 31 cm by 24 cm white nylon fabric bag (12 threads/cm with 0.8 mm openings) over each plant prior to anthesis in 2000. Bags were secured to the ground with plant stakes. Fruit set was compared between bagged and open plants.

Pollen Examination and Comparison

In 1999 and 2000, a pollen reference collection was established by collecting pollen from flowers of plants that were in coincident bloom with *Cypripedium fasciculatum* (*Calochortus subalpinus*, *Calypso bulbosa*, *Ceanothus integerrimus*, *Chimaphila umbellata*, *Cornus nuttallii*, *Cypripedium montanum*, *Fragaria vesca bracteata*, *Iris chrysophylla*, *Myosotis laxa*, *Pachistima myrsinites*, *Senecio* sp., *Trientalis latifolia*, *Whipplea modesta*). Collected pollen was stored in 70% ethanol at the SOU Insect Museum. Samples of pollen collected from plants and pollen found attached to collected diapiiids were examined and compared using a Hitachi® S-2100 scanning electron microscope (SEM). Pollen were first prepared for SEM by acetolysis (Kearns & Inouye 1993) then attached to aluminum stubs with sticky tabs and sputter-coated with gold-palladium on an Emscope® SC500 sputter coater before being viewed by SEM at 1.5 kV. Two other orchids, *Calypso bulbosa* and *Cypripedium montanum*, were found in the research sites and bloomed coincidentally with *C. fasciculatum*. Pollinia from each of these orchids as well as pollinia collected from diapiiids were examined under a stereo dissecting scope (Leica® MZ 75), photographed (SPOT R T Color®, Diagnostic Inc.), measured (SPOT® software, version 4), and compared to *C. fasciculatum* pollinia.

RESULTS

Anthesis and Fruit Set

In all sites and across all years, the numbers of flowers on *Cypripedium fasciculatum* plants ranged from one to seven, with most having two or three flowers. The mean number of flowers per plant in each site was 2.4 in Alexander Gulch, 3.3 in Murphy Gulch, and 3.4 in Butte Falls. Anthesis in flowers on multiple-flowered plants usually occurred simultaneously. In general for all sites and years, floral anthesis began in mid-April and ended no later than early June (FIGURE 2). The date of peak floral anthesis (the date when the largest percentage of flowers was in anthesis) differed by as much as 25 days. Anthesis peaked on 5 May, 7 May, and 26 April in 1999, 2000, and 2001, respectively, at Murphy Gulch; on 21 May and 18 May in 1999 and 2000, respectively, at Alexander Gulch; and on 3 May in 2001 at Butte Falls (FIGURE 2A–F). Anthesis was accelerated on bagged plants compared with open plants at Murphy Gulch in 2000. The total duration of anthesis could not be compared for all sites, because the earliest date of anthesis was not recorded at all sites. The number of days when greater than 50% of the flowers were in anthesis, however, differed by as much as 12 days and as little as one day for all sites and years (FIGURE 2).

Fruit set in the *Cypripedium fasciculatum* populations varied marginally across years ($F = 16.42$; $df = 1, 3$; $P = 0.056$) but did not differ significantly across sites in the same year ($F = 0.001$; $df = 1, 3$; $P = 0.975$) (TABLE 2). In 1999, loss of flowers from other factors (e.g., insect and animal browsing) was minimal and overall, and adjusted fruit set was relatively high at Murphy Gulch (58% and 71.5%, respectively) and at Alexander Gulch (54.7% and 85.4%, respectively) (TABLE 2), suggesting a high rate of pollination. In 2000, overall fruit set in both Murphy Gulch and Alexander Gulch was much lower (14.1% and 20%, respectively). Adjusted fruit set in the same year for open (unbagged) flowers was 43.3% and 27.1% in Murphy and Alexander, respectively, suggesting a lower rate of pollination in 2000 than in 1999. Overall fruit set in bagged (pollinator-excluded) plants was 0.0% at each site in 2000 (TABLE 2). In 2001, both overall and adjusted fruit set was low at Murphy Gulch (17.5% and 36.9%, respectively) and at Butte Falls (8.9% and 17.0%, respectively). Plants at Butte Falls showed the lowest fruit set recorded during the 3-year study (TABLE 2).

Diapiiid Activity, Anthesis, and Fruit Set

Although total number of insects caught in sticky traps was more than three times greater

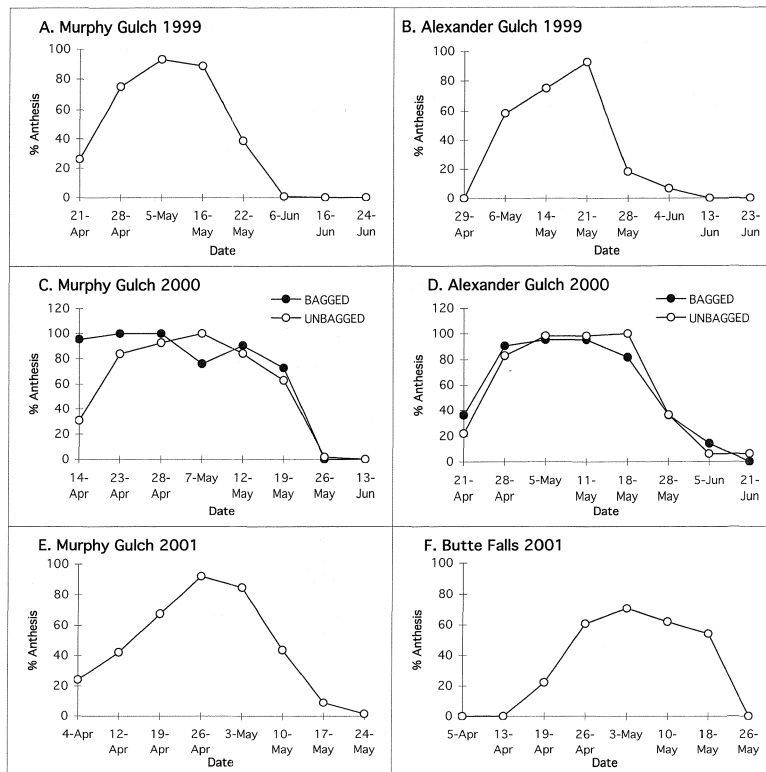


FIGURE 2. Percent of *Cypripedium fasciculatum* flowers in anthesis throughout the blooming period in all sites during 1999, 2000, and 2001.

at Murphy Gulch than at Alexander and ten times higher in 1999 than in 2000 (TABLE 3), the percent of total insects of each taxa caught were similar for sites and years. Except for Alexander Gulch in 2000, a greater number of sciarids, the known host to diapiids, was captured than any

other insect group. Mycetophilids were consistently a minor component of the insect fauna captured in both sites across both years. Other insects captured in both sites included various Diptera (Chironomidae, Culicidae, Tipulidae, Cecidomyiidae, Empididae, Phoridae, and Mus-

TABLE 2. Number of plants, number of flowers, and percent fruit set in *Cypripedium fasciculatum* flowers at each field site in 1999, 2000, and 2001.

Year	Site	Fruit set (%)		Flowers no.	Plants no.
		Overall	Adjusted		
1999	Murphy Gulch	58.0	71.5	169	49
1999	Alexander Gulch	54.7	85.4	75	30
2000	Murphy Gulch				
	Bagged*	0.0	0.0	26	8
	Unbagged	14.1	43.3	92	30
2000	Alexander Gulch				
	Bagged	0.0	0.0	22	10
	Unbagged	20.0	27.1	65	28
2001	Murphy Gulch	17.5	36.9	137	42
2001	Butte Falls	8.9	17.0	282	83

* Bags covering two plants in Murphy Gulch in 2000 were inadequately secured, and data collected from these plants were subsequently removed from the analysis.

TABLE 3. Total number and percent of selected taxa (Diptera, Hymenoptera, and Coleoptera) caught in sticky traps placed at the Murphy Gulch and Alexander Gulch study sites 12 April–24 June 1999 and 7 April–28 May 2000.

Taxonomic group/year	Murphy Gulch		Alexander Gulch	
	No.	%	No.	%
1999				
Total Diptera	2199	60.6	530	58.4
Sciaridae	1521	41.9	243	26.7
Mycetophilidae	41	1.1	8	0.9
Other Diptera	637	17.6	279	30.8
Total Hymenoptera	974	26.9	345	38.0
Diapriidae	237	6.5	84	9.3
Other Hymenoptera	737	20.4	261	28.7
Total Coleoptera	454	12.5	37	0.4
Total Insects	3627		907	
2000				
Total Diptera	212	73.1	69	39.4
Sciaridae	158	54.4	38	21.7
Mycetophilidae	5	1.7	2	1.1
Other Diptera	49	16.9	29	16.6
Total Hymenoptera	61	21.0	79	45.1
Diapriidae	8	2.8	8	4.6
Other Hymenoptera	53	18.3	71	40.6
Total Coleoptera	17	5.9	27	15.4
Total Insects	290		175	

cidae), parasitic Hymenoptera (Ichneumonoidea, Chalcidodea, Cynipoidea, and Proctotrupeoidea), and non-parasitic Hymenoptera (Formicidae, Apoidea, and Symphyta). In 1999, diapriids comprised a large percentage (26–43%) of all parasitic Hymenoptera caught at both sites (TABLE 3). In contrast, only eight diapriids (less than 10% of all parasitic Hymenoptera) were caught at each site in 2000 (TABLE 3). In 2001, when insect activity was monitored throughout the year (22 Jan.–27 Dec.), activity of sciarids, mycetophilids, and diapriids was greatest in the spring (mid-February–early June) with peak activity occurring during orchid bloom (mid-April–early June) (FIGURE 3). Despite similar insect activity inside and outside the plots at each site, overall activity was significantly lower at Butte Falls than at Murphy Gulch.

Although diapriid activity (mean number caught per trap per day) showed a marginally significant negative correlation ($R^2 = 0.585$; $df = 1, 5$; $P = 0.076$) with the duration of anthesis in *Cypripedium fasciculatum*, diapriid activity was not correlated significantly with fruit set ($R^2 = 0.301$; $df = 1, 5$; $P = 0.259$) across all sites and years (TABLE 4).

Pollen Examination and Comparison

Cypripedium fasciculatum pollen grains similar to the pollen of other Cypripedioideae (mo-

nads with no sculpturing) were readily distinguishable from pollen of coincidentally blooming non-orchid plants (Burns-Balogh & Hesse 1988, Newton & Williams 1978, Xi & Chen 1991). Individual pollen grains from *C. fasciculatum* were held together by elastoviscin (Pacini & Hesse 2002) as a mass or pollinium that appeared yellow and smooth under a light microscope (FIGURE 4A). The pollinia of *C. fasciculatum*, which could be dislodged into pieces when mechanically manipulated with a probe, was distinguishable from other coincidentally blooming orchids. *Cypripedium fasciculatum* pollinia measured ca. 0.9 mm by 0.5 mm (FIGURE 4A). *Calypso bulbosa* has distinctively shaped waxy pollinarium, consisting of two pairs of small and large pollinia with its associated viscidium, measured at least twice the size of *C. fasciculatum* (Proctor & Harder 1995, Pacini & Hesse 2002). *Cypripedium montanum* pollinia, while similar in appearance to *C. fasciculatum*, also were larger (ca. 2.0 mm by 1.8 mm) than *C. fasciculatum* pollinia. Diapriid wasps collected in our study typically measured 3–4 mm in total and would not be able to accommodate the larger sized pollinia of either *C. bulbosa* or *C. montanum*.

Cypripedium fasciculatum are diandrous and lack a rostellum and/or viscidium (Proctor et al. 1996). We observed *C. fasciculatum* pollinium separating into two discrete units or massulae,

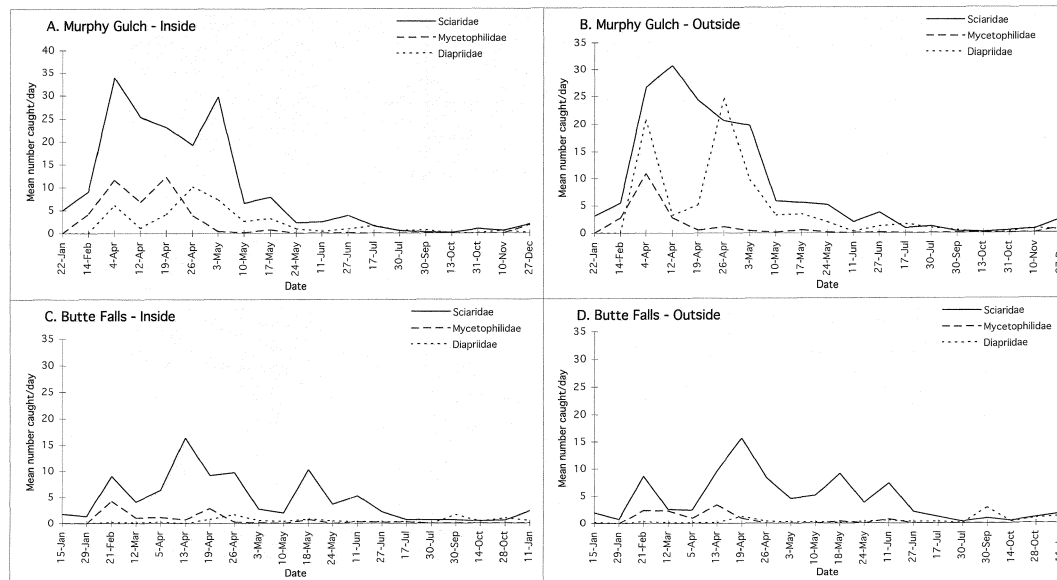


FIGURE 3. The mean daily trap catch of diapiiids, mycetophilids, and sciarids during a 12-month period in 2001.

while still on the anther of a sacrificed flower (FIGURE 4A) (Johnson & Edwards 2000). One or both masses of *C. fasciculatum* pollinia consistently were deposited on the mesothoracic nota of diapiiids (specifically the mesoscutellar hollow and setae of the mesoscutum) (Nixon 1957; FIGURE 4B). A total of 23 diapiiids were collected with *C. fasciculatum* pollinia during this study, 1, 17, and 5 in 1998, 1999, and 2001, respectively. All diapiiids carrying *C. fasciculatum* pollinia were females of an undescribed species in the subfamily Belytinae, genus *Cinetus* (L. Masner pers. comm.), and 15 were deposited as voucher specimens in the Canadian National Museum in Ottawa, Ontario. No other insects were found vectoring *C. fasciculatum* pollinia during this study.

DISCUSSION

Insect pollinated plants, particularly those with disjunct populations, rely on adequate pol-

linator activity (Boyd 1994). A year-long monitoring of diapiiid and diapiiid host activity demonstrated that these insect populations are ubiquitous and synchronized with each other as well as with *Cypripedium fasciculatum* bloom. Diapiiid activity, as measured by the mean number of diapiiids captured per trap per day, varied significantly across the years of this study but not across study sites within the same year. Because fungus gnat larvae and pupae (the hosts for diapiiids) live in the soil, where they depend on adequate moisture and temperatures to survive, the affects of precipitation, temperature, and other general factors on diapiiid hosts likely were responsible (more than site-specific factors such as aspect, slope, elevation, etc.) for differences in diapiiid activity at the study sites across the years.

Although fruit set in *Cypripedium fasciculatum* was not correlated significantly with diapiiid activity across sites or years, fruit set mar-

TABLE 4. Duration of anthesis in *Cypripedium fasciculatum* and diapiiid activity (Number caught/trap/day) at each field site in 1999, 2000, and 2001.

Year	Site	Anthesis (days)	Diapiiids (no./trap/day)
1999	Murphy Gulch	18	1.02
1999	Alexander Gulch	15	0.53
2000	Murphy Gulch	26	0.01
2000	Alexander Gulch	20	0.08
2001	Murphy Gulch	14	0.90
2001	Butte Falls	22	0.10

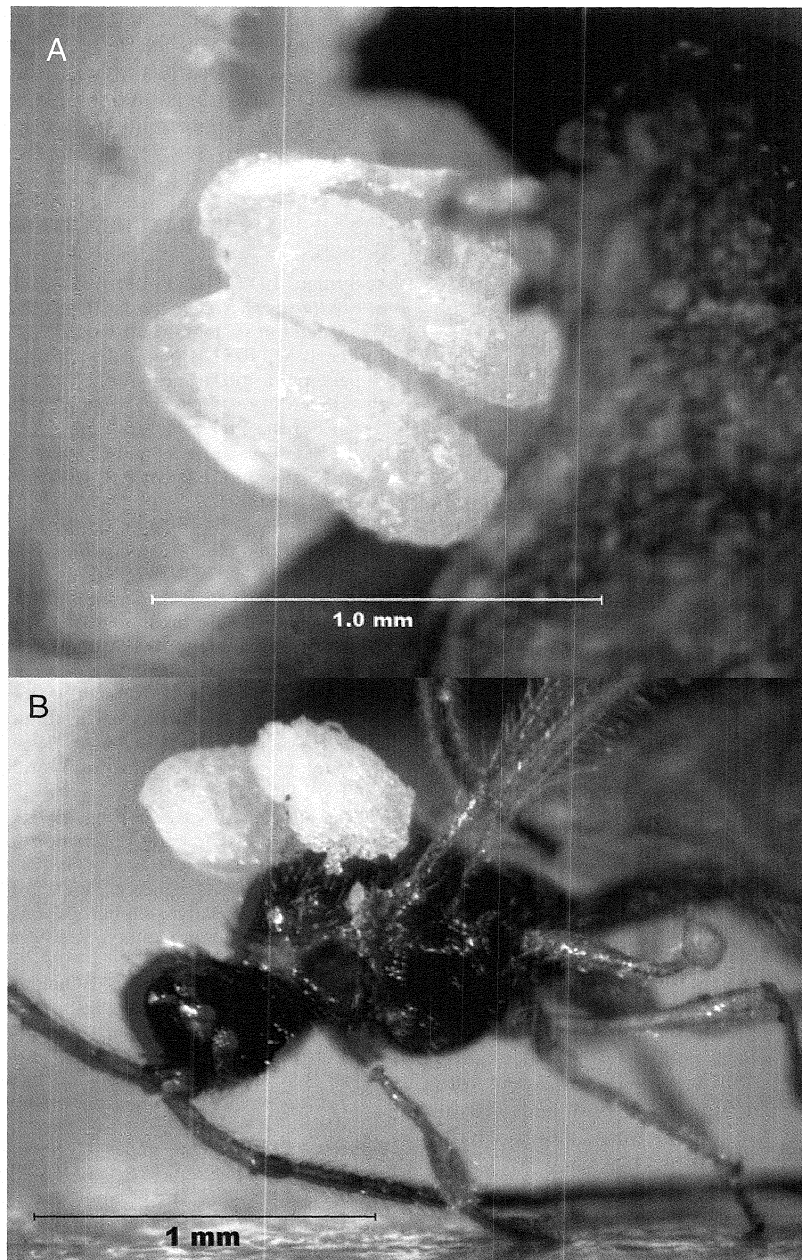


FIGURE 4. **A.** *Cypripedium fasciculatum* pollinium separating into two discrete massulae. **B.** Dorso-lateral view of *Cinetus* sp. (Diapriidae) with *C. fasciculatum* pollinia on its thorax. Photos by C. Ferguson.

ginally differed across years and was greatest in 1999 when diapiiid activity was greatest. The significant loss in plants resulting from animal browsing during our study may have prevented us from accurately assessing fruit set, thus possibly confounding correlation between fruit set

and diapiiid activity. The relatively high fruit sets, those above the purported average of 20% for non-rewarding orchids (Nielson & Wilcock 1998), that we and others (Kipping 1971, Knecht 1996, Lipow et al. 2002) have reported for *C. fasciculatum* suggests that repeated pol-

linator deception is common. The number of visits required before a pollinator learns to avoid a non-rewarding species may have been increased by variations in the color and/or odor of flowers within the same population (Ackerman 1981). *Cypripedium fasciculatum* flowers often show a wide range of colors within the same population.

Differences in the temporal patterns (initial and peak anthesis and duration) of anthesis across sites within a year suggested that site-specific factors (elevation, aspect, and overstory) might influence patterns of *Cypripedium fasciculatum* bloom. Patterns of *C. fasciculatum* bloom also may be influenced by general factors (weather), since the date of peak anthesis (when the largest percent age of *C. fasciculatum* flowers were in anthesis) varied as much as 11 days between years at the same site. Despite differences in the blooming period of *C. fasciculatum*, peak diapiiid activity consistently was synchronized with the date of peak anthesis across sites and years. A marginally significant negative correlation between diapiiid activity and the duration of anthesis in this study may indicate that diapiiids induce floral senescence in *C. fasciculatum*. Proctor & Harder (1995) revealed a similar pollinator-induced senescence in another non-rewarding orchid, *Calypso bulbosa* var. *occidentalis*. Although the presence or absence of pollen on the anthers of plants monitored in our study was noted, we were unable to monitor pollen deposition on the stigma of *C. fasciculatum* without damaging the plant. Therefore, we could not investigate whether pollen removal alone or pollen removal in conjunction with pollen deposition triggers floral senescence in *C. fasciculatum*.

By investigating *Cypripedium fasciculatum* anthesis and fruit set in relationship to pollinator activity, our research addressed one aspect of a complex interaction between a non-rewarding orchid and its parasitic wasp pollinator. To further elucidate the relationship, future studies need to investigate floral cues used by *C. fasciculatum* to lure and trap this unlikely pollinator. We hypothesize that the nature of the floral cue used in this relationship is an odor that mimics the odor of fungus gnats, the host insects of diapiiids. Therefore, future studies will focus on the collection, source, and characterization of odors associated with anthesis in *C. fasciculatum*.

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